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[54] METHODS TO CLONE POLYA MRNA

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[56] References Cited

U.S. PATENT DOCUMENTS

4,683,195 7/1987 Mullis et al. 435/6

OTHER PUBLICATIONS

Liang et al. Science 257:967-971 (1992).

Gilliland et al. in "PCR protocols: A guide to methods and application" pp. 60-69, 1990.

Freifelder, D. "Molecular Biology" pp. 402-404 1983. Sambrook, et al., Molecular Cloning: A Laboratory Manual, Second Edition, Cold Spring Harbor Press, pp. 8.6-8.35 (1989).

Sargent, T. D., "Isolation of Differentially Expressed Genes", 1987, Methods in Enzymology, vol. 152, pp. 423-ff.

Lee, S. W., et al., "Positive Selection of Candidate

Tumor-Suppressor Genes by Subtractive Hybridization", 1991, Proc. Natl. Acad. Sci. USA, vol. 88, pp. 2825-2829.

Walker, G. T., et al., "Isothermal In Vitro Amplification of DNA by a Restriction Enzyme/DNA Polymerase System", 1992, vol. 89, pp. 392-396.

Williams, J., et al., "DNA Polymorphisms Amplified by Arbitrary Primers are Useful as Genetic Markers", 1990, Nucleic Acids Research, vol. 18, pp. 6531-6535.

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[57] ABSTRACT

A method for isolating mRNAs as cDNAs employs a polymerase amplification method using at least two oligodeoxynucleotide primers, one being short with arbitrary sequence and another being either short with arbitrary sequence or being capable of hybridizing to the region near the mRNA polyA tail. The oligodeoxynucleotide that is capable of hybridizing to the region near the polyA tail is used as a primer for reverse transcription of the mRNA and the resultant cDNA is amplified with a polymerase using both oligodeoxynucleotides as a primer set.

18 Claims, 2 Drawing Sheets

